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(71) Applicant *(for all designated States except US)*: PFIZER, INC. [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : COE, Jotham, W. [US/US]; 151 Lamphere Road, Mystic, CT 06355 (US). FLIRI, Anton, F., J. [US/US]; 120 McKinley Avenue, Norwich, CT 06360 (US). KANEKO, Takushi [US/US]; 398 Northwood Drive, Guilford, CT 06437 (US). LARSON, Eric, R. [US/US]; 162 Lantern Hill Road, Mystic, CT 06355 (US).

(74) Common Representatives: RICHARDSON, Peter, C. et al.; Pfizer Inc., 235 East 42nd Street, New York, NY 10017 (US).

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(54) Title: HETEROCYCLIC COMPOUNDS FOR ENHANCING ANTITUMOR ACTIVITY

(57) Abstract

2,6-Diaminopurines, 3,5-diamino-6,7,8,9-tetrahydrobenzo[b]thiophene[2,3-d]pyrimidines and 2,4-diaminothieno[3,2-d]pyrimidines useful as inhibitors of P-glycoproteins and potentiators of chemotherapeutic agents.

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HETEROCYCLIC COMPOUNDS FOR ENHANCING ANTITUMOR ACTIVITY

Technical Field

This invention relates to certain heterocyclic compounds and their use as sensitizers of tumor cells to anticancer agents.

Background Art

In cancer chemotherapy the effectiveness of anticancer drugs is often limited by the resistance of tumor cells. Some tumors such as of the colon, pancreas, kidney and liver are generally innately resistant, and other responding tumors often develop resistance during the course of chemotherapy. The phenomena of multidrug resistance (MDR) is characterized by the tumor cell's cross-resistance to adriamycin, daunomycin, vinblastine, vincristine, daxol, actinomycin D and etoposide. The resistance cells are often associated with overexpression of the mdrl gene. This gene product is a family of 140-220 kd trans-membrane phosphoglycoproteins (P-glycoprotein) which function as an ATP-dependent efflux pump. Thus, it has been postulated that this efflux mechanism keeps the intracellular level of the anticancer drug low, allowing the tumor cells to survive.

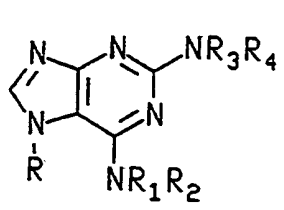
In recent years various substances such as verapamil, nifedipine and diltiazem have been used in in vitro experimental systems to reverse the MDR phenomena. More recently some of these agents have been tested clinically as MDR reversing agents. Little efficacy has been observed with verapamil or trifluoroperazine. Thus, there is a need for an effective MDR reversing agent.

2-Piperazino-4-morpholinothieno[3,2-d]pyrimidines are reported in German Offen. 2,055,085 [CA 77, 88539f (1972)].

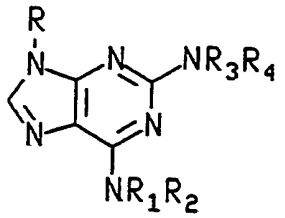
Thienopyrimidines and pyridopyrimidines are claimed as gastric acid secretion inhibitors in European Patent Application 404,356 and 404,355, respectively.

Disclosure of the Invention

The compounds of the present invention are of the formulae



I ,



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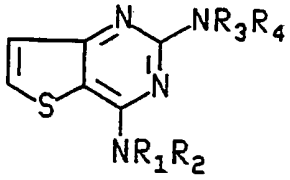
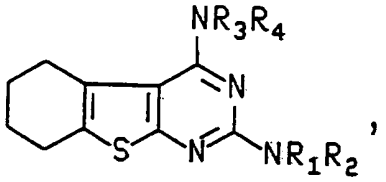
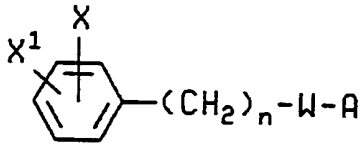


III or



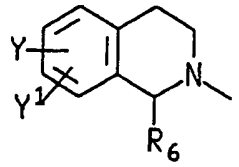
IV ,

and a pharmaceutically acceptable acid addition salt thereof where R is hydrogen, alkyl of one to three carbon atoms or phenylalkyl of seven to ten carbon atoms; R_1 and R_3 are each hydrogen or alkyl of one to three carbon atoms; R_2 and R_4 are each aralkyl of the formula



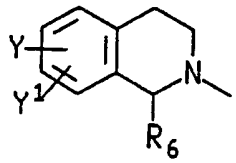
where X and X^1 are each hydrogen, alkyl of one to three carbon atoms, alkoxy of one to three carbon atoms, hydroxy, fluoro, chloro, trifluoromethyl, amino, alkylamino of one to three carbon atoms or dialkylamino of two to six carbon atoms, X and X^1 taken together are methylenedioxy or ethylenedioxy, n is an integer of 0 or 1, W is S, O or a chemical bond and A is alkylene of two to four carbon atoms; and R_1 and R_2 or R_3 and

R_4 when taken togeth r with the nitrogen to which they are attached each form a moiety of the formula

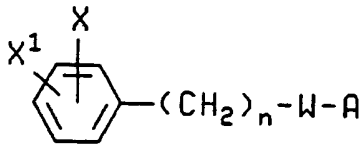


where R_6 is hydrogen, alkyl of one to three carbon atoms or dialkoxyphenylalkyl said alkoxy each of one to three carbon atoms and said alkyl of one to three carbon atoms and Y and Y^1 are each hydrogen, alkyl of one to three carbon atoms, alkoxy of one to three carbon atoms, fluoro, chloro, trifluoromethyl, amino, alkylamino of one to three carbon atoms or dialkylamino of two to six carbon atoms.

A preferred group of compounds are those of formula I, where R is phenylalkyl of seven to ten carbon atoms; R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula

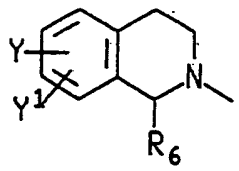


where R_6 is hydrogen and Y and Y^1 are each alkoxy of one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula

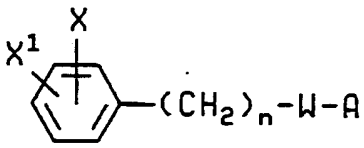


where X and X^1 are each alkoxy of one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene. Especially preferred within this group are the compounds where R is 1-phenylethyl; Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy and where R is benzyl; Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

A second group of preferred compounds are thos of formula II where R is alkyl of on to three carbon atoms or phenylalkyl of seven to ten carbon atoms; R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula

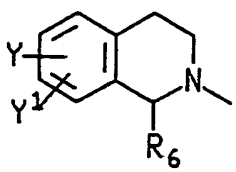


where R_6 is hydrogen or dialkoxyphenylalkyl said alkoxy each of one to three carbon atoms and said alkyl of one to three carbon atoms and Y and Y^1 are each alkoxy of one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula

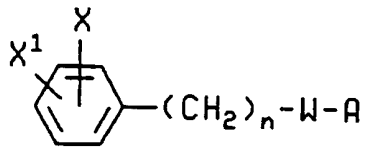


where X and X^1 are each alkoxy of one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene. Especially preferred within this group are the compounds where R is 1-phenylethyl; R_6 is hydrogen, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy, where R is benzyl; R_6 is 3,4-dimethoxybenzyl, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy and where R is methyl; R_6 is 3,4-dimethoxybenzyl, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

A third group of preferred compounds are those of formula III wherein R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



where R_6 is hydrogen and Y and Y^1 are each alkoxy of one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula



where X and X^1 are each alkoxy of one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene. Especially preferred within this group is the compound where Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

The present invention also includes a method of inhibiting a P-glycoprotein in a mammal in need of such treatment which comprises administering to said mammal a P-glycoprotein inhibiting amount of a compound of formulae I-IV. Preferred is the method where the mammal is a human suffering from cancer and said compound is administered before, with or after the administration to said human of an anticancer effective amount of a chemotherapeutic agent.

Also included is a pharmaceutical composition for administration to a mammal which comprises a P-glycoprotein inhibiting amount of a compound of formulae I-IV, a pharmaceutically acceptable carrier and, optionally, an anticancer effective amount of a chemotherapeutic agent.

As previously indicated, the compounds of formulae I-IV form pharmaceutically acceptable acid addition salts. Said pharmaceutically acceptable acid addition salts include, but are not limited to, those with HCl, HBr, HNO_3,H_2SO_4, H_3PO_4, CH_3SO_3H, $C_6H_5SO_3H$, CH_3CO_2H, gluconic acid, tartaric acid, maleic acid and succinic acid. In the case of those compounds of the formulae I-IV which contain a further basic nitrogen, it will, of course, be possible to form diacid addition salts (e.,g., the dihydrochloride) as well as the usual monoacid addition salt.

As one skilled in the art recognizes, compounds of formulae I-IV have the potential for containing asymmetric carbon atoms. All these potential isomers are considered within th scope of the present invention.

Detailed Description

Compounds of the present invention are prepared by reacting a 2,6-dichloropurine, 3,5-dichlorothieno[2,3-d]pyrimidine or 2,4-dichlorothieno[3,2-d]pyrimidine with the requisite amine, R_1R_2NH.

In a more detailed description of the procedure, one molar amount of the dichloro compound and one molar amount of the amine, R_1R_2NH, as the hydrochloride salt are reacted in a water immiscible solvent, such as methylene chloride, containing two molar amounts of a tertiary amine, such as triethylamine. The reaction is usually complete in 3-24 hours when conducted at room temperature.

The 6-chloro group of the 2,6-dichloropurine is the more reactive while the 5- and 4-chloro substituents of the thieno[2,3-d]pyrimidine and thieno[3,2-d]pyrimidine are, respectively, the most reactive.

On completion of the reaction, the reaction mixture is quenched in water and the product isolated by concentration of the water immiscible solvent. Purification of the product can be carried out by recrystallization or column chromatography.

Alternately, the reaction can be carried out in a water miscible solvent, such as dimethylacetamide. In such cases the reaction mixture, on completion, is added to water and the product filtered or extracted.

The isolated intermediate is then reacted with the requisite amine, R_3R_4NH, in a reaction-inert solvent. In practice, one mole of the mono-chloro compound is reacted with one mole of amine, R_3R_4NH, in a highly polar solvent such as 2-(2-ethoxyethoxy)-ethanol containing one mole of a high boiling amine, such as diisopropylethylamine. The reaction temperature is 160-170°C with a reaction time of about 72 hours.

The reaction mixture is cooled to room temperature, diluted with methylene chloride and chromatographed on silica gel. The isolated product is converted to an appropriate salt, for example the hydrochloride salt, by adding it to a methanolic solution of hydrogen chloride. Further purification can be carried out by recrystallization.

Generation of the free base from an acid addition salt can readily be carried out by treating an aqueous solution or suspension of the salt with at least one equivalent of an organic or inorganic bas followed by extraction of the fr e base product with a water immiscible solvent such as ethyl acetate or methylene chlorid . Removal of the solvent gives th desired base.

Compounds of formulae I-IV are inhibitors of the functions of P-glycoprotein, particularly human mdr 1 protein or P-glycoprotein related and membrane associate proteins which are participating in the transport of xenobiotics or proteins across membranes e.g., cell membranes of eukariotic and proeukariotic origin e.g., pmfdr, however not exclusive or restricted to these examples.

Compounds included in general formulae I-IV are useful in combination chemotherapy of cancer, malaria, viral infections such as AIDS, in therapy of septic shock syndrome or inflammation and may be useful in enhancing of the xenobiotics limited due to the presence of P-glycoprotein or P-glycoprotein related functional proteins. Compounds of formulae I-IV increase the activity/efficacy of adriamycin, daunomycin, etoposide, epipodophyllotoxin congeners, actinomycin D, emetin, daxol, vincristine, vinblastine, chloroquine, antracycline antibiotics and of drugs which are structurally and functionally related to the above mentioned examples, in particular, when the activity of these drugs has been shown to be limited due to the presence and function of P-glycoprotein, e.g. human mdr 1 protein or P-glycoprotein related proteins.

The compounds of the present invention are evaluated as potentiators of chemotherapeutic agents using a Cellular Drug Retention Assay. This assay was designed to study the effect of compounds on cellular retention of radiolabeled drug. In this case 14C-adriamycin retention by multidrug resistant human carcinoma cells, KBV1, is measured.

KBV1 cells are routinely grown in tissue culture as monolayers in DMEM high glucose medium containing 1 ug/ml vinblastine, 10% heat inactivated fetal calf serum and supplemented with Glutamine, Pen-strep and Garamycin.

The assay protocol (described below) should be applicable with minor modifications, to a wide variety of cell lines grown in tissue culture.

Assay Protocol:

(1) Seed replicate 6-well tissue culture plates with 1.2x10E6 cells per 2 ml per well in absence of Vinblastine;

(2) Incubate 24 hours at 37 degrees in humidified incubator (5% CO2);

(3) Aspirate off the spent media and overlay monolayers with 2 ml/well of fresh medium that is 2 uM in Adriamycin (2 uM unlabeled Adriamycin + 20000 cpm of 14C-Adr) and the test agent at concentrations varying from 0 to 100 uM;

(4) Following incubation for 3 hours at 37 degrees in humidified incubator, remove media and wash monolayers twice with 2 ml of ice cold buffered saline;

(5) Detach monolayers using 0.5 ml of trypsin/EDTA, collect detached cells and transfer to scintillation vial. Rinse wells once with 0.5 ml of buffered saline and add to same vial containing cells;

(6) Add 5 ml of Beckman Ready-Safe scintillation fluid to vial, vortex and determine radioactivity per sample using a scintillation counter (10 minutes per sample);

(7) For background control: pre-incubate monolayers at 4 degrees for 15 minutes then remove media and add fresh ice-cold media containing Adr (see step 3). Following incubation for 3 hours at 4 degrees remove media and wash monolayers twice with 2 ml ice-cold buffered saline, then proceed as in step 5;

(8) Results are expressed as T/C and ED3x values as defined below:

T/C = pmoles Adr per 10E6 cells treated with test agent/

ED3x = concentration of test agent that produces a 3 fold increase in cellular accumulation of radiolabeled Adr, i.e. T/C = 3.

Calculation

Specific cpm = [sample cpm - background cpm]

Specific activity = [cpm/total conc. of Adr]

pmoles Adr = [specific cpm/specific activity]

pmoles Adr per 10E6 cells = [(pmoles Adr per well/number of cells per well) x 10E6 cells]

As previously mentioned, compounds of the present invention and salts thereof are useful in potentiating the anticancer effects of chemotherapeutic agents. Such agents can include adriamycin, daunomycin, aclacinomycin A, actinomycin C, actinomycin D, mithramycin, vinblastine, maytansine, bruceantin, homoharintonin, anguindin, neocarcinostatin, mitomycin C and anthramycin.

The compounds of the present invention can be administered with, 24 hours before or up to 72 hours after the administration of the chemotherapeutic agents. When administered with said agents, they can be taken either separately or coadministered in the same formulation.

Th compounds of th present invention wh ther taken separately or in combination with an anti-cancer agent, are generally administered in the form of pharmaceutical compositions comprising at least on of the compounds of formula

I-IV and optionally a chemotherapeutic agent, together with a pharmaceutically acceptable vehicle or diluent. Such compositions are generally formulated in a conventional manner utilizing solid or liquid vehicles or diluents as appropriate to the mode of desired administration: for oral administration, in the form of tablets, hard or soft gelation and, for parenteral administration, in the form of injectable solutions of suspensions, and the like.

For use in the potentiation of anticancer agents in a mammal, including man, a compound of formulae I-IV is given in an amount of about 0.5-100 mg/kg/day, in single or divided doses. A more preferred dosage range is 2-50mg/kg/day, although in particular cases, at the discretion of the attending physician, doses outside the broader range may be required. The preferred route of administration is generally oral, but parenteral administration (e.g. intramuscular, intravenous, intradermal) will be preferred in special cases, e.g., where oral absorption is impaired as by disease or where the patient is unable to swallow.

The present invention is illustrated by the following examples, but is not limited to the details or scope thereof.

EXAMPLE 1

2-(3,4-Dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)purine hydrochloride

A. 2-chloro-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)purine

A mixture of 5.67 g of 2,6-dichloropurine, 6.89 g of 1,2,3,4-tetrahydroisoquinoline hydrochloride and 6 g of triethylamine in 40 ml of dimethylacetamide was stirred under a nitrogen atmosphere at room temperature for 3.5 hours. The mixture was poured into 500 ml of water and stirred for 30 minutes. The solids were filtered, washed with water, pressed dry and stirred in hot methanol for 1 hour. The suspension was filtered while hot and the solids dried, 9.85 g (95% yield), m.p. 271-276°C dec.

B. 2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)purine hydrochloride

A mixture of 692 mg of the product of Example 1A, 362 mg of 3,4-dimethyl-phenethylamine and 258 mg of diisopropylethylamine in 6 ml of 2-(2-ethoxyethoxy)-ethanol was stirred under a nitrogen atmosphere at 165°C for 5 hours. The reaction mixture was cooled, diluted with chloroform and the solids filtered. The filtrate was loaded on 90 g of silica gel/chloroform and eluted with 2% methanol chloroform. The

fractions containing the product w re combined and concentrated to dryness, 84 mg. Treatment of the residue with 1N hydrogen chloride in methanol followed by recrystallization from methanol gave 61 mg of the desired product, m.p. 152-154°C.

EXAMPLE 2

2-(3,4-Dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-9-methylpurine hydrochloride

A. 2-chloro-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-9-methylpurine

A suspension of 1.76 g of the product of Example 1A, 930 mg of potassium carbonate and 950 mg of methyl iodide in 100 ml of dimethylsulfoxide was warmed until the purine was dissolved. The reaction was cooled to room temperature and stirred overnight. The mixture was poured over ice, the pH adjusted to 5 with acetic acid and the product extracted with methylene chloride. The extract was washed with water, dried over sodium sulfate and concentrated in vacuo to a yellow oil. The residue was chromatographed on silica gel using from 0% methanol in chloroform to 2% methanol in chloroform (V:V). The fractions containing the product were combined and concentrated to dryness. The residual foam was triturated with methanol to give 2.09 g of the desired product, m.p. 182-184°C.

B. 2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-9-methylpurine hydrochloride

Following the procedure of Example 1B and starting with 1.44 g of the product of Example 2A, 724 mg of 3,4-dimethoxyphenethylamine and 516 mg of diisopropylethylamine in 2 g of 2-(2-ethoxyethoxy)ethanol gave 115 mg of the desired product, m.p. 179-181°C.

EXAMPLE 3-7

Employing the procedure of Example 1B and starting with the appropriate reagents, the following compounds were prepared:

2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-9-benzylpurine hydrochloride, m.p. 152-154°C;

2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-7-benzylpurine hydrochloride, m.p. 139-141°C;

2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-7-methylaminopurine hydrochloride, m.p. 159-164°C;

2-(3,4-dimethoxyph nethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-7-(1-phenylethylamino)purine hydrochloride, m.p. 128-132°C;

2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-9-(1-phenylethylamino)purine hydrochloride, m.p. 108-114°C;

2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-1-[3,4-dimethoxybenzyl]-6,7-dimethoxyisoquinol-2-yl)-7-benyzlpurine hydrochloride, m.p. 139-141°C;

2-(3,4-dimethoxyphenethylamino)-6-(1,2,3,4-tetrahydro-1-[3,4-dimethoxybenzyl]-6,7-dimethoxyisoquinol-2-yl)-9-methylpurine hydrochloride, m.p. 148-150°C.

EXAMPLE 8

2-(3,4-Dimethoxyphenethylamino)-4-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)thieno[3,2-d]pyrimidine hydrochloride

A. 2-chloro-4-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)thieno[3,2-d]pyrimidine

A mixture of 1.381 g of 2,4-dichlorothieno[3,2-d]pyrimidine, 1.55 g of 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride and 1.41 g of triethylamine in 40 ml of dimethylacetamide was stirred at room temperature for 72 hours. The reaction mixture was poured into 300 ml of water and the solids filtered, dried and recrystallized from methanol, 1.7 g, m.p. 173-175°C.

B. 2-(3,4-dimethoxyphenethylamino)-4-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)thieno[3,2-d]pyrimidine hydrochloride

A mixture of 1.08 g of the product of Example 8A, 543 mg of 3,4-dimethoxyphenethylamine and 387 mg of diisopropylethylamine in 1.25 g of 2-(2-ethoxyethoxy)-ethanol was stirred at 170°C for 24 hours under nitrogen. The reaction was cooled to room temperature and diluted with 5 ml of chloroform. The resulting solution was chromatographed on silica gel using from 0% methanol in chloroform to 2% methanol in chloroform as the eluent. The fractions containing the product were combined and concentrated to an orange oil. Treatment of the oil with 15 ml of a 1N solution of hydrogen chloride in methanol gave 1.04 g of the desired product, m.p. 210-212°C.

EXAMPLE 9

3-(3,4-Dimethoxyphenethylamino)-5-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-6,7,8,9-tetrahydrobenzothieno[2,3-d]pyrimidine hydrochloride

A. 3-chloro-5-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-6,7,8,9-tetrahydrobenzothieno[2,3-d]pyrimidine

A solution of 623 mg of 3,5-dichloro-6,7,8,9-tetrahydrobenzothieno[2,3-d]pyrimidine, 554 mg of 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride and 4 ml of triethylamine in 40 ml of methylene chloride was stirred at room temperature under nitrogen for 15 hours. An additional 275 mg of the appropriate tetrahydroisoquinoline hydrochloride and 1 ml of triethylamine were added and stirring continued for an additional 9 hours. The reaction mixture was diluted with 100 ml of methylene chloride and extracted with 1N hydrochloric acid (3 x 75 ml), water (1 x 75 ml) and a brine solution (1 x 75 ml). The organic phase was dried over sodium sulfate and concentrated to an oil. The residue oil was dissolved in methanol and the resulting precipitated solids filtered and dried, 740 mg, m.p. 158-160°C.

B. 3-(3,4-dimethoxyphenethylamino)-5-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-6,7,8,9-tetrahydrobenzothieno[2,3-d]pyrimidine hydrochloride

A solution of 666 mg of the product of Example 9A, 290 mg of 3,4-dimethoxyphenethylamine and 206 mg of diisopropylethylamine in 800 mg of 2-(2-ethoxyethoxy)-ethanol was heated at 170°C under nitrogen for 24 hours. The reaction mixture was cooled to room temperature, diluted with 3 ml of chloroform and chromatographed on 40 g of silica gel using chloroform as the eluent. The fractions containing the product were combined, concentrated in vacuo and the residue chromatographed on 25 g of silica gel in an 18 inch by 25 mm column using chloroform as the eluent and collecting 6 ml fractions. Fractions 9-20 were combined, concentrated and the residue added to 1N methanolic hydrogen chloride. The solids were filtered and dried, 211 mg, m.p. 195-198°C.

PREPARATION A

2,6-Dichloro-7-benzylpurine and 2,6-dichloro-9-benzylpurine

To a suspension of 4.56 potassium carbonate and 5.67 g of 2,6-dichloropurine in 40 ml of dimethylsulfoxide was added, 5.64 g of benzylbromide. Th mixtur was stirred for 45 minutes under nitrogen at room temperature and was then poured onto crushed ice. The pH of the mixture was adjusted to 5 with acetic acid and extracted

with methylene chloride (2 x 400 ml). The combined extracts were washed with water (6 x 400 ml), and brine (1 x 400 ml), dried over sodium sulfate and evaporated. The residue was chromatographed on silica gel using chloroform-methanol (9:1-V:V) as the eluent to give 3.59 g of 2,6-dichloro-9-benzylpurine, m.p. 152-152.5°C and 1.32 g of 2,6-dichloro-7-benzylpurine, m.p. 151-151.5°C.

PREPARATION B

2-Chloro-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-7-benzylpurine

A mixture of 950 mg of 2,6-dichloro-7-benzylpurine, 780 mg of 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride and 700 mg of triethylamine in 80 ml of dimethylacetamide was stirred for 72 hours at room temperature under nitrogen. The reaction mixture was poured into water (300 ml) and the resulting solids filtered, dried and recrystallized from methanol, 1.25 g, m.p. 195-197°C.

In a similar manner, 1.4 g of 2,6-dichloro-9-benzylpurine gave 1.87 g of 2-chloro-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-9-benzylpurine, m.p. 151-153°C.

PREPARATION C

2,6-Dichloro-7-methylpurine and 2,6-dichloro-9-methylpurine

In a manner similar to Preparation A, 9.52 g of 2,6-dichloropurine, 7.65 g of potassium carbonate and 7.86 g of methyl iodide in 65 ml of dimethylsulfoxide gave 1.54 g of 2,6-dichloro-7-methylpurine and 4.7 g of 2,6-dichloro-9-methylpurine.

PREPARATION D

2-Chloro-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-7-methylpurine

A solution of 1.44 g of 2,6-dichloro-7-methylpurine, 1.63 g of 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride and 1.5 g of triethylamine in 25 ml of methylene chloride was stirred at room temperature under nitrogen for 15 hours. The reaction mixture was washed with a 1N hydrochloric acid solution (3 x 150 ml), water (3 x 150 ml) and a brine solution (1 x 100 ml), and then dried over sodium sulfate. Removal of the solvent left a residue which was chromatographed on 150 g of silica gel, 1.3 g.

PREPARATION E

2,5-Dichloro-7-(1-phenylethyl)purine and 2,6-dichloro-9-(1-phenylethyl)purine

Using the sam gen ral procedure of Preparation A, 4.12 g of 2,6-dichloro-purine, 3.32 g of potassium carbonat and 4.44 g of 1-bromoethylbenzen gav 830

mg of 2,6-dichloro-7-(1-phenylethyl)purine and 1.3 g of 2,6-dichloro-9-(1-phenylethyl)purine.

PREPARATION F

2-Chloro-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-7-(1-phenylethyl)purine

Employing the procedure of Preparation B, 730 mg of 2,6-dichloro-7-(1-phenylethyl)purine, 573 mg of 1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline hydrochloride and 505 mg of triethylamine in 25 ml of dimethylacetamide gave 430 mg of the desired intermediate.

Similarly, 2-Chloro-6-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl)-9-(1-phenylethyl)purine was prepared in 76% yield.

PREPARATION G

2-Chloro-6-(1,2,3,4-tetrahydro-1-[3,4-dimethoxybenzyl]-6,7-dimethoxyisoquinol-2-yl)-9-methylpurine

Using the procedure of Preparation D, 2.01 g of 2,6-dichloropurine, 3.76 g of 1,2,3,4-tetrahydro-1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride and 4 g of triethylamine in 20 ml of methylene chloride gave 2.31 g of the titled product.

Similarly, 1.42 g of 2,6-dichloro-9-benzylpurine, 1.93 g of 1,2,3,4-tetrahydro-1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride and 2.12 g of triethylamine in 40 ml of methylene chloride gave 1.98 g of 2-Chloro-6-(1,2,3,4-1-[3,4-dimethoxybenzyl]-6,7-dimethoxyisoquinol-2-yl)-9-benzylpurine.

PREPARATION H

3,5-Dichloro-6,7,8,9-tetrahydrobenzo[2,3-d]pyrimidine

1. 3,5-dihydroxy-6,7,8,9-tetrahydrobenzo[2,3-d]pyrimidine

A mixture of 22.53 g of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate and 39.04 g of urea were fused at 180-190°C for 3 hours under nitrogen. The mixture was cooled to room temperature and treated with 600 ml of 6N potassium hydroxide solution. The suspension was filtered and the cooled filtrate adjusted to pH 2 with concentrated hydrochloric acid. The precipitated solids were filtered and slurred in refluxing water. The suspension was filtered while hot and the solids dried to give the titled product.

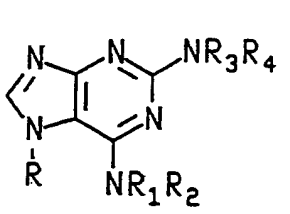
2. 3,5-dichloro-6,7,8,9-tetrahydrobenzo[2,3-d]pyrimidine

The product of Preparation H-1 (4.44 g) was added to 40 ml of phosphorous oxychloride and th reaction mixture refluxed for 72 hours. The reaction was cooled

and added carefully to 500 ml of warm water. The cooled mixture was extracted with chloroform (3 x 500 ml) and the combined extracts washed with water (2 x 500 ml) and a brine solution (1 x 300 ml). After drying over sodium sulfate, the solvent was removed in vacuo and the residue chromatographed as the eluent. The fractions containing the product were combined and concentrated. The residue was recrystallized from methanol, m.p. 175-178°C.

CLAIMS

1. A compound of the formula



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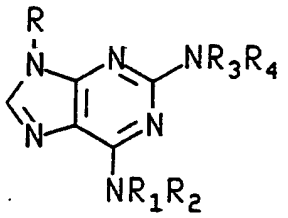

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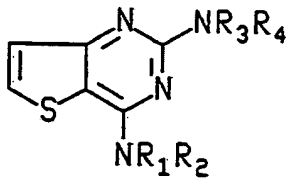

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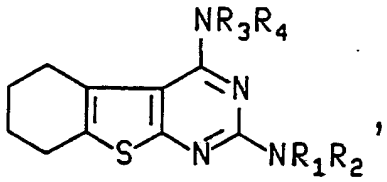
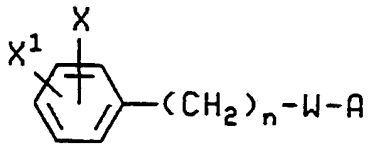
or



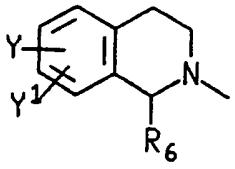
IV

and the pharmaceutically acceptable acid addition salt thereof, wherein R is hydrogen, alkyl having one to three carbon atoms or phenylalkyl having seven to ten carbon atoms; R_1 and R_3 are each hydrogen or alkyl having one to three carbon atoms; R_2 and R_4 are each aralkyl of the formula



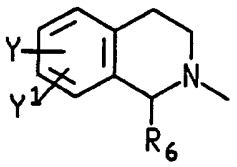
where X and X^1 are each hydrogen, alkyl having one to three carbon atoms, alkoxy having one to three carbon atoms, hydroxy, fluoro, chloro, trifluoromethyl, amino, alkylamino having one to three carbon atoms or dialkylamino having two to six carbon atoms, X and X^1 taken togeth r are methylenedioxy or ethylenedioxy, n is an integer of 0 or 1, W is S, O or a chemical bond and A is alkylen having two to four carbon

atoms; and R_1 and R_2 or R_3 and R_4 when taken together with the nitrogen to which they are attached each form a moiety of the formula

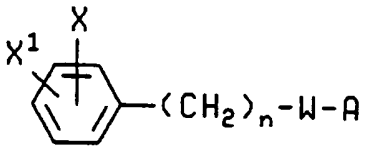


where R_6 is hydrogen, alkyl having one to three carbon atoms or dialkoxyphenylalkyl said alkoxy each having one to three carbon atoms and said alkyl having one to three carbon atoms and Y and Y^1 are each hydrogen, alkyl having one to three carbon atoms, alkoxy having one to three carbon atoms, fluoro, chloro, trifluoromethyl, amino, alkylamino having one to three carbon atoms or dialkylamino having two to six carbon atoms.

2. A compound of claim 1, formula I, where R is phenylalkyl having seven to ten carbon atoms; R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



where R_6 is hydrogen and Y and Y^1 are each alkoxy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula

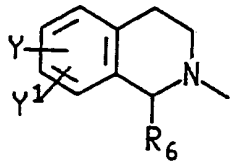


where X and X^1 are each alkoxy having one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

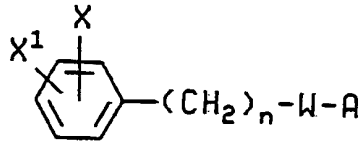
3. The compound of claim 2, where R is 1-phenyl thyl; Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

4. The compound of claim 2, where R is benzyl; Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

5. A compound of claim 1, formula II, where R is alkyl having one to three carbon atoms or phenylalkyl having seven to ten carbon atoms; R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



where R_6 is hydrogen or dialkoxyphenylalkyl said alkoxy each having one to three carbon atoms and said alkyl having one to three carbon atoms and Y and Y^1 are each alkoxy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula



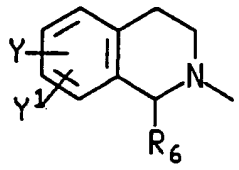
where X and X^1 are each alkoxy having one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

6. The compound of claim 5, where R is 1-phenylethyl; R_6 is hydrogen, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

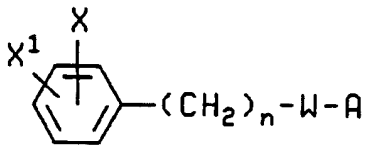
7. The compound of claim 5, where R is benzyl; R_6 is 3,4-dimethoxybenzyl, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

8. The compound of claim 5, where R is methyl; R_6 is 3,4-dimethoxybenzyl, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

9. A compound of claim 1, formula III, where R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



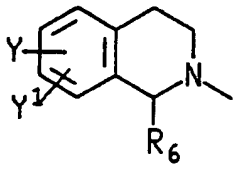
where R_6 is hydrogen and Y and Y^1 are each alkoxy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula



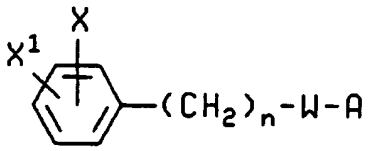
where X and X^1 are each alkoxy having one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

10. The compound of claim 9, where Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

11. A compound of claim 1, formula IV, where R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



where R_6 is hydrogen and Y and Y^1 are each alkoxy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula



where X and X^1 are each alkoxy of one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

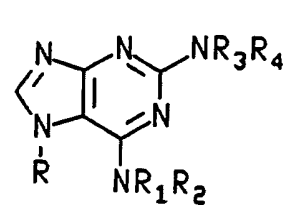
12. The compound of claim 11, where Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

13. A method of inhibiting a P-glycoprotein in a mammal in need of such treatment which comprises administering to said mammal a P-glycoprotein inhibiting amount of a compound according to claim 1.

14. A method of claim 13, wherein the mammal is a human suffering from cancer and said compound is administered before, with or after the administration to said human of an anticancer effective amount of a chemotherapeutic agent.

15. A pharmaceutical composition for administration to a mammal which comprises a p-glycoprotein inhibiting amount of a compound of claim 1, a pharmaceutically acceptable carrier and, optionally, an anticancer effective amount of a chemotherapeutic agent.

16. A process for preparing a compound of the formula



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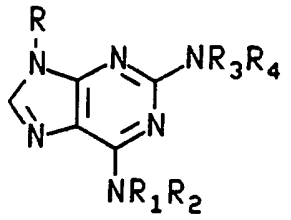

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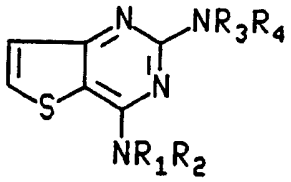

III

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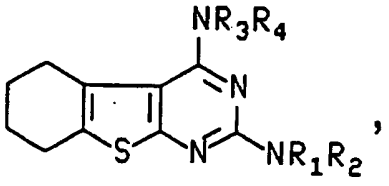
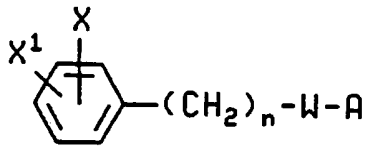


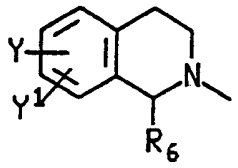
IV

and the pharmaceutically acceptable acid addition salt thereof, wherein R is hydrogen, alkyl having one to three carbon atoms or phenylakyl having seven to ten carbon atoms; R_1 and R_3 are each hydrogen or alkyl having one to three carbon atoms; R_2 and R_4 are each aralkyl of the formula



where X and X^1 are each hydrogen, alkyl having one to three carbon atoms, alkoxy having one to three carbon atoms, hydroxy, fluoro, chloro, trifluoromethyl, amino alkylamino having one to three carbon atoms or diakylamino having two to six carbon atoms, X and X^1 taken together are methylenedioxy or ethylenedioxy, n is an integer of 0 or 1, W is S, O or a chemical bond and A is alkylene having two to four carbon

atoms; and R_1 and R_2 or R_3 and R_4 when taken together with the nitrogen to which th y are attached each form a moiety of the formula



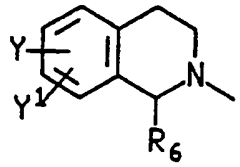
where R_6 is hydrogen, alkyl having one to three carbon atoms or dialkoxyphenylalkyl said alkoxy each having one to three carbon atoms and said alkyl having one to three carbon atoms and Y and Y^1 are each hydrogen, alkyl having one to three carbon atoms, alkoxy having one to three carbon atoms, fluoro, chloro, trifluoromethyl, amino, alkylamino having one to three carbon atoms or dialkylamino having two to six carbon atoms, comprising:

(a) for compounds of formula I or II, reacting the appropriate 2-chloro-6-aminopurine derivative with R_3R_4NH HCl salt and a tertiary amine in a reaction-inert solvent at a reaction temperature of 160-170° C until the reaction is substantially complete, and optionally forming a pharmaceutically acceptable salt thereof by methods known per se;

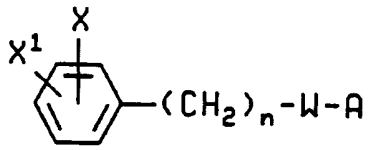
(b) for compounds of formula III, reacting the appropriate 2-chloro-4-amino-thieno[3,2-d]pyrimidine derivative with R_3R_4NH HCl salt and a tertiary amine in a reaction-inert solvent at a reaction temperature of 160-170° C until the reaction is substantially complete, and optionally forming a pharmaceutically acceptable salt thereof by methods known per se; or

(c) for compounds of formula IV, reacting the appropriate 3-chloro-5-amino-6,7,8,9-tetrahydrobenzo[b]thiophene[2,3-d]pyrimidine derivative with R_3R_4NH HCl salt and a tertiary amine in a reaction-inert solvent at a reaction temperature of 160-170° C until the reaction is substantially complete, and optionally forming a pharmaceutically acceptable salt thereof by methods know per se.

17. A process of claim 16 wherein, for compounds of formula I, R is phenylakyl having seven to ten carbon atoms; R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



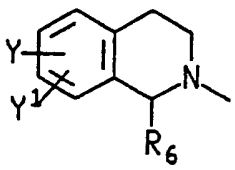
where R_6 is hydrogen and Y and Y^1 are each alkoxy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula



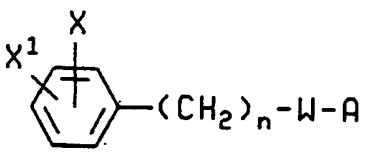
where X and X^1 are each alkoxy having one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

18. A process of claim 17 wherein, R is 1-phenylethyl or benzyl; Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

19. A process of claim 16 wherein, for compounds of formula II, R is alkyl having one to three carbon atoms or phenylalkyl having seven to ten carbon atoms; R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



where R_6 is hydrogen or dialkoxyphenylalkyl said alkoxy each having one to three carbon atoms and said alkyl having one to three carbon atoms and Y and Y^1 are each alkoxy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula

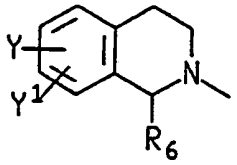


where X and X^1 are each alkoxy having one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

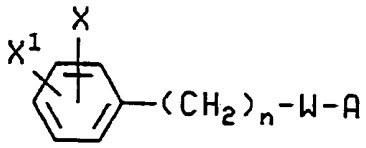
20. A process of claim 19 wherein, R is 1-phenylethyl; R_6 is hydrogen, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4 methoxy.

21. A process of claim 19 wherein, R is benzyl or methyl; R_6 is 3,4-dimethoxybenzyl, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

22. A process of claim 16 wherein, for compounds of formula III, R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



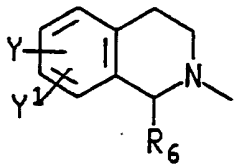
where R_6 is hydrogen and Y and Y^1 are each alkoxy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula



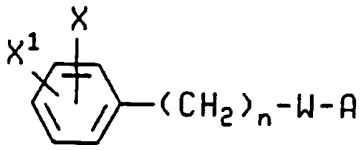
where X and X^1 are each alkoxy having one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

23. A process of claim 22 wherein, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

24. A process of claim 16 wherein, for compounds of formula IV, R_1 and R_2 taken together with the nitrogen to which they are attached form a moiety of the formula



where R_6 is hydrogen and Y and Y^1 are each alk xy having one to three carbon atoms; R_3 is hydrogen; and R_4 is aralkyl of the formula



where X and X^1 are each alkoxy of one to three carbon atoms, n is 0, W is a chemical bond and A is ethylene.

25. A process of claim 24 wherein, Y is 6-methoxy and Y^1 is 7-methoxy; and X is 3-methoxy and X^1 is 4-methoxy.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/09554

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D473/16; C07D495/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0 300 726 (MERCK) 25 January 1989 see page 67 - page 71; claims	1-25
A	DE,A,2 200 764 (DR. KARL THOMAE) 12 July 1973 see page 26 - page 30; claims	1-25
A	FR,A,2 135 294 (DR. KARL THOMAE) 15 December 1972 see page 37 - page 40; claims	1-25
A	US,A,3 016 378 (JOSEF ROCH) 9 January 1962 *Complete specification*	1-25
A	FR,A,1 281 229 (DR. KARL THOMAE) 4 December 1961 *Complete specification*	1-25

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° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 FEBRUARY 1993	-4. 03. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LUYTEN H.W.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP,A,0 103 114 (SANKYO) 21 March 1984 see page 44 - page 48; claims	1-25
A	EP,A,0 212 535 (BOEHRINGER MANNHEIM) 4 March 1987 see claims	1-25
A	EP,A,0 404 356 (SMITH KLINE BECKMAN INTERCREDIT B.V.) 27 December 1990 cited in the application see page 16 - page 19; claims	1-25
A	CHEMICAL ABSTRACTS, vol. 77, no. 13, 25 September 1972, Columbus, Ohio, US; abstract no. 88539f, page 468 ;column L ; cited in the application see abstract & US,A,3 763 156 (EDWARD WOITUN) 2 October 1973	1-25

INTERNATIONAL SEARCH REPORT

PCT/US92/09554

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
"Remark: Although claims 13,14 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition."

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9209554 SA 67882

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 25/02/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0300726	25-01-89	AU-B-	601862	20-09-90
		AU-A-	1923088	27-01-89
		JP-A-	1104074	21-04-89
		US-A-	5057517	15-10-91
DE-A-2200764	12-07-73	None		
FR-A-2135294	15-12-72	AT-B-	318619	11-11-74
		AU-A-	4181572	08-11-73
		BE-A-	783044	06-11-72
		DE-A-	2121950	23-11-72
		GB-A-	1393161	07-05-75
		NL-A-	7206041	07-11-72
		US-A-	3888851	10-06-75
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US-A-3016378		None		
FR-A-1281229		None		
EP-A-0103114	21-03-84	JP-C-	1694401	17-09-92
		JP-B-	3063552	01-10-91
		JP-A-	59042387	08-03-84
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		AU-A-	1698583	26-01-84
		US-A-	4562193	31-12-85
EP-A-0212535	04-03-87	DE-A-	3529497	26-02-87
		JP-A-	62045588	27-02-87
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EP-A-0404356	27-12-90	JP-A-	3017083	25-01-91